DOI : 10.5958/0974-0112.2020.00007.9

Indian J. Hort. 77(1), March 2020: 88-93

Influence of drought stress and brassinosteroid on growth and physio-biochemical characteristics of apple plants

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ABSTRACT

The experiment was conducted to study the effect of drought stress and brassinosteroid on growth and physio-biochemical responses of one-year-old apple plants of 'Super Chief' and 'Red Chief' varieties. These plants were treated with foliar application of brassinosteroid 0.05 ppm and 0.10 ppm, three days prior to 15 days and 30 days of drought stress. The maximum reduction in growth parameters were registered by plants subjected to 30 days of drought stress as compared to 15 days of drought stress. The transpiration rate owed significant reduction under drought stress. Osmoregulating substances like free amino acid, total soluble sugar content and antioxidant enzyme such as catalase activity enhanced during drought stress which helped in enhancing drought tolerance. Maximum reduction of soil moisture content was registered in 30 days of drought stress over 15 days. However, brassinosteroid at both concentrations could help in maintaining vital growth and physio-biochemical processes under drought stress conditions. Foliar spray of brassinosteroid (0.05 ppm) before the imposition of stress can pave the way to minimize the deleterious effects of drought stress on apple plants.

Key words: *Malus domestica*, brassinosteroid, physiological, osmoregulating substances, catalase.

INTRODUCTION

Apple is a major fruit crop grown by the farmers of Himachal Pradesh and occupies a significant position in the horticultural wealth (Kumari and Thakur, 8). In Himachal Pradesh, apple is mainly grown under rainfed conditions, where rainfall is not well distributed throughout the year. Since, most of the rainfall is received during monsoon and winter seasons, there is a very lesser amount of rains during vital periods of plant growth and development (Singh *et al.,* 13). 'Super Chief' and 'Red Chief' both are super type varieties of apple having good fruit quality and production. Their chilling requirement is lower as compared to other standard varieties. Climate changes highly influencing chilling period due to reduced rainfall during the critical period of growth stage directly influence plant growth and development. In the temperate region particularly during summer months the increase in the transpiration and evaporation rates, impose drought stress in many crop plants, affecting almost all plant functions (Chandel, 6). In these conditions, drought stress causes a serious reduction in plant growth and development (Tuna *et al.,* 15). The severity of drought stress has a great impact on the physiological and biochemical process of plants. Plant responses to water stress are usually screened on the level of selected physiological parameters such as water potential, relative water content, stomatal reactions, photosynthesis and transpiration rate, which have been proven to be good indicators of drought (Atkinson *et al.,* 4). In recent studies, osmolyte accumulation, altered active oxygen species (AOS) and antioxidative enzymatic responses have also been proposed under drought stress.

Phytohormones have been implicated in modulating plant response to desiccation stress. Brassinosteroids (BRs) are a class of polyhydroxysteroids that have been recognized as the sixth class of plant hormones. One of the most promising roles of brassinosteroid is their ability to confer resistance to a wide array of abiotic stresses (Bajguz and Hayat, 5). Keeping in this view, this study was conducted to study the effect of water stress and brassinosteroid on growth, water relations and physio-biochemical changes responses of one-year-old apple cvs.'Super Chief' and 'Red Chief'.

MATERIALS AND METHODS

The experiment was laid out during 2016 (February,9- June, 31) at an elevation of 1250 m above mean sea level at 30° 51'N latitude and 76° 11'E longitude in the Department of Fruit Science, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh. One-year-old plants grafted on *Malus baccata* of 'Super Chief' and 'Red Chief' were planted in plastic pots of 85 cm × 30 cm (diameter × height) and subjected to four treatments namely

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T1- Control (without treatment), T_2- Drought stress, T_3- Brassinosteroid 0.05 ppm and T_4- Brassinosteroid 0.10 ppm, followed by 15 days and 30 days of drought stress. Observations regarding growth parameters, *viz*. plant height, leaf area, root/shoot fresh and dry weight and root to shoot ratio were recorded according to standard procedures. The transpiration rate and stomatal conductance were recorded with LICOR-6200 portable photosynthesis system. The total free amino acid content in leaves of the same age was estimated by the method described by Lee and Takahasi (9). The method of Nelson (11) was adopted for the determination of total soluble sugars. Catalase activity was estimated according to Abohatem *et al.* (1). Soil moisture content was recorded by using soil moisture meter HH2. The experiment was laid out in Completely Randomized Design (CRD) with four replications. All data were subjected to two way factorial ANOVA carried out using the SPSS computer package (SPSS Inc. USA).

RESULTS AND DISCUSSION

It is evident from the data presented in Table 1 to 4 that plant growth parameters were influenced significantly both by different treatments as well as durations in one-year-old plants of both varieties *viz.,* Super Chief and Red Chief. The maximum plant height was recorded for 30 days of drought stress, it was lowest in plants subjected to 15 days of drought stress. Pretreatment with foliar application of 0.05 ppm brassinosteroid followed by drought stress shows significant increases in plant height in 'Super Chief' (78.28 cm) and 'Red Chief'(68.13cm) over control. The leaf area is considered an important criterion to measure drought stress. Leaf area was registered maximum (46.46 cm^2 and 42.37 cm^2) for 30 days and minimum (43.46 cm^2 and 39.79 cm^2) for 15 days of drought stress in both varieties'Super Chief' and 'Red chief'. Interactions between durations and treatments were found significant in respect of plant height and insignificant for leaf area. These findings are in conformity with those of Misger and Kumar (10). The apple plants responded similarly to drought stress on the accumulation of fresh weight and dry weight of shoot, root and root: shoot. In both the cultivars, higher and lower shoot fresh weight, shoot dry weight, root fresh weight and root dry weight were registered under 30 days and 15 days of imposed drought stress, respectively. However, pre-treatment of brassinosteroid at both the concentrations (0.05 and 0.10 ppm) before the imposition of water stress could help in maintaining the higher fresh and dry weight of shoot and root over drought stress. There was a significant reduction in root: shoot under drought stress. The drought durations and treatments interactions were found significant in both the varieties. Similarly, Alizadeh *et al.* (2) also reported that drought stress causes significant reductions in plant growth parameters such as vegetative shoot growth, trunk area, leaf area, leaf number, root number, wet and dry weight of leaf, leaf water potential, relative water content, relative water loss and shoot diameter.

Drought also resulted in a decrease of transpiration rate. The data presented in Table 4 revealed that the transpiration rate decreased with the decrease in soil moisture content in both varieties 'Super Chief' and 'Red Chief'. The results showed that maximum transpiration rate (25.60 mmol/m^2/s and 23.99 mmol/m^2/s) reported under 15 days of drought stress and

Table 1. Effect of drought stress and brassinosteroid (BR) on plant height and leaf area of apple varieties.

Treatments	Super Chief			Red Chief			Super Chief			Red Chief		
	Plant height (cm)						Leaf area (cm^2)					
	Durations											
	15 days	30 days	Mean	15 days	30 days	Mean	15 days	30 days	Mean	15 days	30 days	Mean
Control	80.10	89.93	85.02	69.95	79.89	74.92	45.57	50.29	47.93	41.92	46.43	44.17
Drought stress	74.12	77.59	75.90	64.05	67.44	65.75	42.10	43.74	42.92	38.15	39.79	38.97
BR (0.05 ppm)*	76.67	79.90	78.28	66.51	69.75	68.13	44.56	46.84	45.70	40.61	42.89	41.75
BR (0.10 ppm)*	74.52	78.04	76.28	64.37	67.89	66.13	42.43	44.96	43.69	38.48	40.36	39.42
Mean	76.37	81.37	78.87	66.22	71.24	68.73	43.66	46.46	45.06	39.79	42.37	41.08
$CD_{(0.05)}$ Duration (D)		0.63			0.63			0.08			0.09	
Ttreatment (T)		0.44			0.44			0.06			0.06	
D×T		0.89			0.89			NS			NS	

*Followed by drought stress

Table 2. Effect of drought stress and brassinosteroid (BR) on shoot fresh weight and shoot dry weight of apple varieties.

Treatments	Super chief			Red Chief			Super Chief			Red Chief		
	Shoot fresh weight (g)						Shoot dry weight (g)					
	Durations											
	15 days	30 days	Mean	15 days	30 days	Mean	15 days	30 days	Mean	15 days	30 days	Mean
Control	75.11	84.93	80.02	65.07	74.98	70.02	27.11	29.51	28.31	26.41	28.51	27.46
Drought stress	69.08	72.48	70.78	59.08	62.48	60.78	24.17	22.17	23.17	21.42	23.51	22.47
BR (0.05 ppm)*	71.54	74.79	73.10	61.55	64.79	63.17	25.16	26.89	26.03	24.52	25.51	25.01
BR (0.10 ppm)*	69.41	72.92	71.16	59.41	62.92	61.16	24.51	24.96	24.74	23.98	22.73	23.35
Mean	71.28	76.28	73.78	61.27	66.29	63.78	25.24	25.88	25.56	24.54	24.61	24.57
$CD_{(0.05)}$ Duration (D)		0.61			0.62			0.04			0.03	
Ttreatment (T)		0.43			0.44			0.03			0.02	
D×T		0.87			0.87			0.06			0.04	

*Followed by drought stress

Table 3. Effect of drought stress and brassinosteroid (BR) on root fresh weight and root dry weight of apple varieties.

Treatments	Super Chief			Red Chief			Super Chief			Red Chief		
	Root fresh weight (g)						Root dry weight (g)					
	Durations											
	15 days	30 days	Mean	15 days	30 days	Mean	15 days	30 days	Mean	15 days	30 days	Mean
Control	55.76	65.58	60.67	45.86	55.84	50.85	25.74	30.63	28.19	16.11	21.03	18.57
Drought stress	49.72	53.12	51.42	39.72	43.12	41.42	22.37	18.90	20.63	12.87	9.40	11.13
BR (0.05 ppm)*	52.18	55.43	53.80	42.18	45.43	43.80	23.84	25.46	24.65	14.34	15.96	15.15
BR (0.10 ppm)*	50.05	53.56	51.80	40.05	43.56	41.80	22.77	24.05	23.41	13.27	14.55	13.91
Mean	51.93	56.92	54.42	41.95	46.99	44.47	23.68	24.76	24.22	14.15	15.23	14.69
$CD_{(0.05)}$ Duration (D)		0.61			0.61			0.31			0.30	
Treatment (T)		0.43			0.43			0.22			0.21	
D×Tq		0.87			0.87			0.44			0.42	

*Followed by drought stress

Table 4. Effect of drought stress and brassinosteroid (BR) on root: shoot and transpiration rate of apple varieties.

Treatments	Super Chief			Red Chief			Super Chief			Red Chief		
	Root: Shoot						Transpiration rate (mmol/ m^2/s)					
	Durations											
	15 days	30 days	Mean	15 days	30 days	Mean	15 days	30 days	Mean	15 days	30 days	Mean
Control	0.874	0.632	0.753	0.584	0.819	0.701	29.11	30.06	29.58	27.48	28.60	28.04
Drought stress	0.956	0.968	0.962	0.741	0.676	0.708	25.85	22.50	24.17	24.20	20.85	22.52
BR (0.05 ppm)*	0.941	0.974	0.957	0.761	0.780	0.771	27.65	25.21	26.43	26.00	23.56	24.78
BR (0.10 ppm)*	0.932	0.942	0.937	0.747	0.764	0.755	26.62	24.63	25.62	24.97	22.98	23.97
Mean	0.926	0.879	0.902	0.708	0.760	0.734	27.31	25.60	26.45	25.66	23.99	24.83
$CD_{(0.05)}$ Duration (D)		0.006			0.006			0.62			0.62	
Treatment (T)		0.004			0.004			0.44			0.44	
D×T		0.008			0.008			0.87			0.88	

*Followed by drought stress

minimum transpiration rate (27.31 mmol/m^2/s and 25.66 mmol/m^2/s) register under 30 days drought stress in respective cultivars. It is noteworthy that pre-treatment of brassinosteroid at both the concentration (0.05ppm and 0.10 ppm) before the imposition of water stress could help in maintaining higher transpiration rate than that observed in stressed plants. The interactions between durations and treatments were found significant in respect of transpiration rate. Chandel and Chauhan (7) have also observed a decrease of transpiration rate under soil moisture stress in apple plants.

The biochemical characteristics such as accumulation of osmolytes enhance the drought tolerance of apple plants. Therefore total free amino acid and total soluble sugar content was recorded. The data are given in Table 5 showed the significant enhancement in total free amino acid and total soluble sugars in the leaves of unstressed and drought-stressed plants of both varieties 'Super Chief' and 'Red Chief'. The maximum increase in total free amino acid (9.07 mg g^{-1} and 9.96 mg g^{-1}) and total soluble sugar (55.53 mg g-^{1} dry wt and 46.01 mg g-^{1} dry wt) were registered under 30 days of drought stress and minimum (6.27 mg $g^{-1}$and 6.80 mg g^{-1}) total free aminoacid and total soluble sugar (39.64 mg g^{-1} dry wt.) and 55.53 mg g^{-1} dry wt.) were reported under 15 days of drought stress in respective cultivars i.e. 'Super Chief' and 'Red Chief'. Foliar application of brassinosteroid at both the concentrations (0.05 ppm and 1.00 ppm) before the imposition of water stress could help in maintaining osmolytes activity higher than that observed in stressed plants. The interactions between durations and treatments were also found significant in respect to total free amino acid and total soluble sugars. Likewise, Pinheiro *et al.* (12) observed that the increased accumulation of amino acid content was valued in response to osmotic stress since an early accumulation of free amino acid content could contribute to a greater level of stress tolerance. Soluble sugars may function as a typical osmoprotectant, stabilizing cellular membranes and maintaining turgor pressure. Therefore, soluble sugars have been specified as potential osmoregulatory since elevated sugar levels relative to control in stressed plants may contribute to the turgor maintenance.

The data presented in Table 6 revealed that antioxidant enzyme such as catalase activity significantly increases as the duration of drought stress increases. Catalase activity was registered maximum (0.266 A_{230}/g and 0.269$_{230}$/g) under 30 days of drought stress and minimum (0.225$_{230}$/g and 0.228 $_{230}$/g) under 15 days of drought stress in respective cultivars Super Chief' and 'Red Chief'. However, it is observed that pants pre-treated with brassinosteroid 0.05 and 0.10 ppm could maintain the significantly higher activity of catalase in stressed plants. This is in conformity with those of Anuradha and Rao (3) who observed that exogenous application of brassinosteroid increased scavenging activity and removal of reactive oxygen species as reflected by elevated activities of antioxidant enzymes, catalase and peroxidase. Soil moisture content under unstressed and stressed conditions has been expressed in Table 6. Maximum soil moisture content was recorded for control in Super Chief (30.50%) and Red Chief (30.66 %) after 30 days, whereas minimum soil moisture content

Table 5. Effect of drought stress and brassinosteroid (BR) on total free amino acids and total soluble sugars of apple varieties.

Treatments	Super Chief			Red Chief			Super Chief			Red Chief		
	Total free amino acids (mg g^{-1})						Total soluble sugars (mg g^{-1} dry wt.)					
	Durations											
	15 days	30 days	Mean	15 days	30 days	Mean	15 days	30 days	Mean	15 days	30 days	Mean
Control	4.88	4.95	4.91	4.95	5.05	5.00	32.31	32.41	43.59	31.58	31.89	31.73
Drought stress	8.82	15.15	11.99	9.37	16.51	12.94	43.89	67.35	55.62	43.25	53.52	48.38
BR (0.05 ppm)*	5.43	7.75	6.59	6.04	8.83	7.44	40.54	47.60	44.07	39.90	46.96	43.43
BR (0.10 ppm)*	5.97	8.45	7.21	6.85	9.43	8.14	41.83	52.32	47.08	41.19	51.68	46.44
Mean	6.27	9.07	7.67	6.80	9.96	8.38	39.64	55.53	47.59	38.98	46.01	42.49
$CD_{(0.05)}$ Duration (D)		0.02			0.02			0.75			0.63	
Treatment (T)		0.02			0.01			0.53			0.44	
D×T		0.03			0.03			1.06			0.89	

*Followed by drought stress

Table 6. Effect of drought stress and brassinosteroid (BR) on catase activity and soil moisture content of apple varieties.

Treatments	Super Chief			Red Chief			Super Chief			Red Chief		
	Catalase activity(A_{230}/g)						Soil moisture content (%)					
	Durations											
	15 days	30 days	Mean	15 days	30 days	Mean	15 days	30 days	Mean	15 days	30 days	Mean
Control	0.180	0.187	0.184	0.178	0.181	0.179	30.39	30.61	30.50	30.66	30.67	30.66
Drought stress	0.210	0.250	0.230	0.215	0.254	0.234	12.50	7.28	9.89	12.33	7.13	9.73
BR(0.05 ppm)*	0.270	0.315	0.292	0.274	0.323	0.298	16.49	8.50	12.50	16.46	8.38	12.42
BR (0.10 ppm)*	0.241	0.311	0.276	0.245	0.318	0.281	15.69	8.08	11.88	15.62	8.04	11.86
Mean	0.225	0.266	0.246	0.228	0.269	0.248	18.77	13.61	16.19	18.78	17.56	16.17
$CD_{(0.05)}$ Duration (D)		0.002			0.002			0.15			0.21	
Treatment (T)		0.001			0.001			0.11			0.15	
D×T		0.002			0.003			0.22			0.29	

*Followed by drought stress

9.82% and 9.73 % was recorded for 30 days of stress in 'Super Chief' and 'Red Chief', respectively. Brassinosteroid 0.05 and 0.10 ppm showed higher soil moisture content than water stress alone. The interactions between durations and treatments were found significant for soil moisture content. The results are in consistence with the findings of Thakur *et al.,* (14) who reported that moisture content on 25th days of stress declined and ranged between 7.26-8.01% among various cultivars of olive.

From the present investigation, it may be concluded that water stress for 15 and 30 days led to a reduction in growth characteristics with a higher reduction in 30 days stressed plants. Pre-treatment with brassinosteroid both at 0.05 and 0.10 ppm concentration before imposing water stress helped in minimizing the deleterious effects of water stress on apple cv. "Super Chief and Red Chief" however, 0.05 ppm was more effective in counteracting the effect of water stress. Plant indices i.e. plant height, leaf area, root/shoot fresh and dry weight and root to shoot ratio were showed reductions under water stress conditions. Physiological indices i.e. transpiration rate was showed reductions under water stress conditions. However, brassinosteroid at both concentrations could help in maintaining vital plant growth and development. Relative accumulation of osmoregulating substances e.g. total amino acids and total soluble sugars and activities of anti-oxidant enzyme namely catalase were following growth characteristics for different stress durations and treatments. Foliar spray of brassinosteroid (0.05 ppm) before the imposition of stress can pave the way in minimizing the deleterious effects of water stress on apple plants.

ACKNOWLEDGEMENTS

Authors are highly thankful for the facilities and funds provided by Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan 173 230 (H.P.) India.

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Received : August, 2018; Revised : January, 2020;
Accepted : February, 2020